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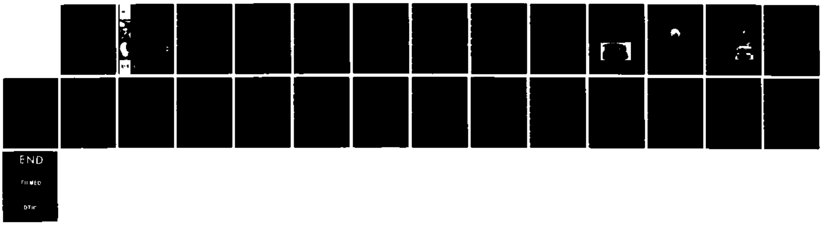

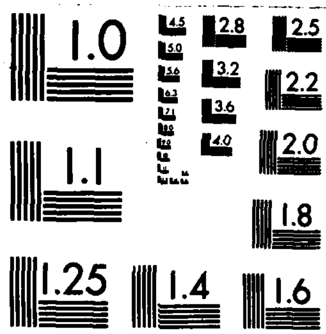

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MORTALITY OF THIRD INSTAR *SAMEODES ALBIGUTTALIS* LARVAE DUE TO CONSTANT LOW TEMPERATURE EXPOSURES IN THE LABORATORY

by

R Michael Stewart

Environmental Laboratory

DEPARTMENT OF THE ARMY
Waterways Experiment Station, Corps of Engineers
PO Box 631, Vicksburg, Mississippi 39180-0631

and

Fred G. Howell

Department of Biology
University of Southern Mississippi
Hattiesburg, Mississippi 39401

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The objective of this project was to determine the proportion of third instar *Sameodes albiguttalis* larvae that will be killed when exposed to a constant low temperature. Test temperatures studied were +6°, +4°, +2°, -2°, -4°, and -6°C.

No significant mortality resulted from exposures of either +6° or +4°C. Exposures to temperatures of +2°C or lower resulted in significant mortality.

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20. ABSTRACT (Continued).

Mortality at -4° and -6°C was similar and approximately twice that observed at -2°C.

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Preface

This report describes a study to establish baseline information on the overwintering capability of *Sameodes albiguttalis* (Warren) (Lepidoptera: Pyralidae). Funds were provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the Aquatic Plant Control Research Program (APCRP) at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The principal investigator for the work was Mr. R. Michael Stewart, WES, who prepared this report. He was assisted in this study and report preparation by Dr. Fred G. Howell, University of Southern Mississippi, Hattiesburg, Miss.

The research was monitored by Dr. Dana R. Sanders, Sr., Mr. Russell F. Theriot, Mr. Edwin A. Theriot, and Dr. Alfred F. Cofrancesco, Jr., of the WES Environmental Laboratory (EL), Wetlands and Terrestrial Habitat Group (WTHG). The study was conducted under the general supervision of Dr. John Harrison, Chief, EL; Dr. Conrad J. Kirby, Jr., Chief, Environmental Resources Division; and the direct supervision of Dr. Hanley K. Smith, WTHG. Mr. J. Lewis Decell was Program Manager of the APCRP at WES.

COL Tilford C. Creel was Commander and Director of the WES during conduct of the study and preparation of this report. Mr. F. R. Brown was Technical Director.

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MORTALITY OF THIRD INSTAR *SAMEODES ALBIGUTTALIS* LARVAE DUE TO CONSTANT LOW TEMPERATURE EXPOSURES IN THE LABORATORY

Introduction

Start

Background

1. This project was designed to establish baseline information on the overwintering capability of the moth *Sameodes albiguttalis* (Warren) (Lepidoptera: Pyralidae). This moth has proven to be an effective agent in controlling the growth of waterhyacinth in locations where populations of the moth have remained established for several consecutive growing seasons. In areas where establishment success has been limited, the effectiveness of *S. albiguttalis* has been less noticeable.

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2. ~~In South America~~, native populations of *S. albiguttalis* occur throughout the natural range of waterhyacinth. DeLoach and Cordo (1978) reported the occurrence of five generations in a population of *S. albiguttalis* near Buenos Aires, Argentina, from September 1975 through September 1976. Results of monthly sampling during this study showed a decrease in the synchrony of developmental stages of this population throughout the growing season until late fall (June 1976). During the winter months (June through August), however, an increase in the generation time (fifth generation) coincided with a considerable increase in the proportion of larvae in field collections. This reportedly resulted in a fairly synchronized progression in this fifth generation to the pupal stage in early spring (September 1976). Although it has not been verified in the United States, the occurrence of this resynchronization of developmental stage in the overwintering generation should result in large numbers of adults emerging within a short time period to begin the first generation of the succeeding growing season. This would be advantageous from a control viewpoint because it should result in large numbers of first generation larvae in the field at the onset of waterhyacinth regrowth.

3. The extended length of this larval stadium suggested the possibility of a facultative diapause, an overwintering "strategy" referred to as oligopause in the classification proposed by Mansingh (1971). DeLoach* doubted that a larval diapause had occurred in the Argentine population and suggested

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* Personal Communication, 1979, C. J. DeLoach, Biological Control of Weeds Laboratory, Hurlingham, Buenos Aires Prov. Argentina.

that the sampling technique had excluded other developmental stages from collection during winter months. Cofrancesco* has observed active larvae of various instars during winter months in Louisiana. These and similar findings in Florida suggest that larvae tolerate winter adversity via temporary quiescent periods.

4. Even though Brou** has captured adult *S. albiguttalis* in light traps during winter months in Louisiana, thereby verifying that other developmental stages do overwinter in the southeastern United States, the reported predominance of larvae in the overwintering population in Argentina (and their subsequent development to first generation adults of the next growing season) suggests the extreme importance of the overwintering success of the larval stage to population survival. Furthermore, Center and Durden (1981) concluded that low winter temperatures alone might possibly have been responsible for establishment failures of *S. albiguttalis* populations at northern Florida release sites.

Scope

5. For these reasons the scope of this research was directed at determining the effects of low temperature exposures on *S. albiguttalis* larvae. Since no established populations of this moth are located in close proximity to Hattiesburg, Miss., acquisition of larvae logistically prevented testing each of the five instars. Because there is no larval diapause in this species, there is no suggestion that one instar will be better adapted than others by specialized physiological processes (e.g. glycogen conversion to glycerol) (Hanec 1966, Mansingh 1974) to withstand below-freezing temperatures. Looking strictly from a physical standpoint, we chose to test the effects of low temperature exposures on third instar larvae. This was decided because it is the middle instar, and as such its body-surface-to-body-volume ratio should be in the center of the range of this variable for all five instars. This consideration was based on the findings of Salt (1956) who investigated the relationship of body size to tissue freezing susceptibility in insects.

6. The specific objective of this project was to determine the proportion of third instar *S. albiguttalis* larvae that will be killed when

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* Personal Communication, 1981, Alfred F. Cofrancesco, Jr., U. S. Army Engineer Waterways Experiment Station, Vicksburg, Miss.

** Personal Communication, 1981, Vernon A. Brou, Louisiana Entomologist, Edgard, La.

subjected to a constant low temperature. The results of this work should be useful as a baseline from which to direct future research on the overwintering capability of this species.

Materials and Methods

Larval rearing

7. Insects used in this study were the progeny of adults reared from pupae shipped to the University of Southern Mississippi (USM), Hattiesburg, Miss., from either field populations in Florida or from a greenhouse facility at the U. S. Army Engineer Waterways Experiment Station (WES) in Vicksburg, Miss. Pupae were partially dissected from the petioles of waterhyacinth and placed in groups of three or four into disposable petri dishes (100 × 15 mm) which were fitted with a piece of moistened filter paper to prevent desiccation. The petri dishes were collectively placed inside a rectangular glass container (20 cm × 18 cm × 10 cm) sealed with cellophane. These were left at ambient room temperature (20° to 25°C) and natural photoperiod until adults emerged. Emergence usually continued for 4 to 5 days after collection.

8. The adults were transferred to a glass aquarium containing several waterhyacinth pseudolaminae (Figure 1). Pseudolaminae were cut approximately

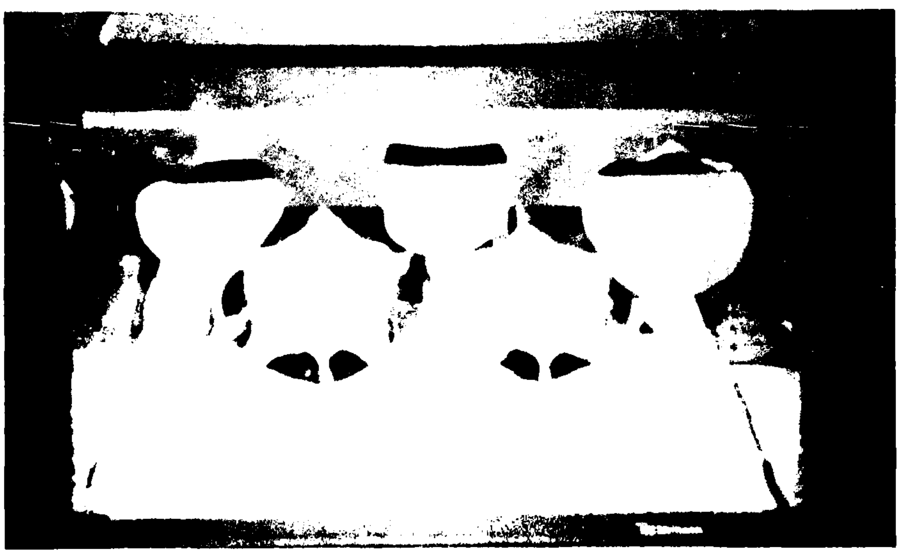

Figure 1. Ovipositional chamber, with prepared waterhyacinth pseudolaminae, used for obtaining eggs of *S. albiguttalis*

10 to 15 cm below the isthmus, and the petioles were submerged in small jars containing water to prevent wilting. Following the methods of Center and Durden (1981), a small incision in the petiole was made at the isthmus, and the epidermis was pulled away apically to expose underlying tissues for oviposition by females.

9. After oviposition, leaves containing eggs were left in the aquarium until the eggs began to darken. When this occurred, leaves were cut at the isthmus and placed singly in petri dishes (Figure 2). Eclosion usually began 4 to 6 days after oviposition.

Figure 2. Plastic petri dish containing moist filter paper and the prepared portion of a waterhyacinth pseudolamina on which female *S. albiguttalis* oviposited

10. Groups of approximately 100 newly emerged larvae were placed on fresh leaf material in separate petri dishes. These were transferred with a camel-hair brush to fresh leaf material each day for the first 3 days. On the fourth day, larvae were placed in groups of approximately 30 larvae per petri dish. These groups were transferred to fresh leaf material evey 2 days. At each change of leaf material, the head capsule widths of one group were measured and larval instar was determined based on the criteria established by Center and Durden (1981). When the third instar stage was reached by a majority of the larvae, they were placed in groups of 20 larvae per petri dish and allowed to feed for approximately 12 hr. Low temperature exposure tests were conducted on these groups.

Test chamber

11. A FREAS 818 Series environmental chamber was used to house the low temperature tests. Within this unit, an 11.4-ℓ aquarium, topped with a styrofoam lid, was emersed in an ethylene glycol solution inside an 18.9-ℓ aquarium. This smaller aquarium was used as the exposure chamber (Figure 3). A LAUDA Model T-1 circulating water pump, equipped with a heating element, was placed in the ethylene glycol solution to maintain uniform temperatures. Temperature in the environmental chamber was always thermostatically set several degrees below the desired exposure temperature. Therefore, the heating unit in the circulating pump maintained the actual test temperature.

12. Temperatures in the exposure chamber were monitored with a YSI 42SC telethermometer. The probe was inserted into an empty petri dish through a hole cut in the top plate. The dish was placed haphazardly inside the exposure chamber. A mercury thermometer was inserted through the styrofoam lid and positioned near the center of the chamber as a reference for the telethermometer. Maximum temperature fluctuations rarely exceeded +0.5°C and never exceeded +1.0°C. Fluctuations were corrected by either addition of ice to the chamber bath or by adjusting the heating unit. Temperatures were monitored at least every 30 min for the shortest exposures and by a strip-chart event recorder during extended absences during longer exposures.

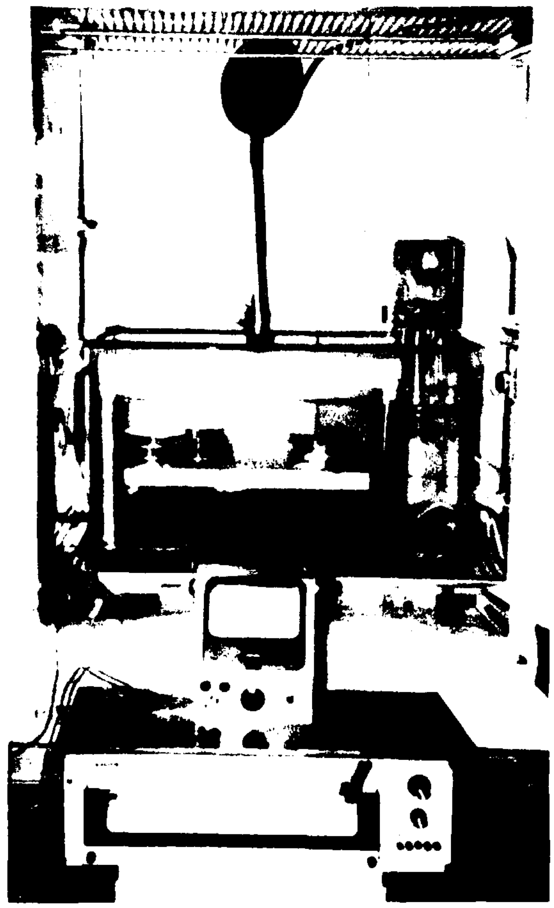

Figure 3. Exposure chamber and monitoring devices used to evaluate mortality of third instar *S. albiguttalis* larvae at constant low temperatures

Experimental design

13. Groups of third instar larvae were exposed to temperatures of +6°, +4°, +2°, -2°, -4°, or -6°C. The exposure duration for each treatment to these temperatures is presented in Table 1. For each temperature, at least

five durations were tested. Exposure durations were selected which would hopefully result in greater than 1 percent mortality in the shortest exposure and less than 99 percent mortality in the longest exposure. Each treatment consisted of three replications of 20 larvae. Additionally, three replications of 20 larvae were held at room temperature in total darkness as a control for each test temperature. Tests and controls were conducted in total darkness because the light in the environmental chamber, when turned on, made it difficult to maintain stable temperatures in the exposure chamber. All tests and control treatments were started at the same time for a given temperature.

14. For each test, treatment replicates were removed from the exposure chamber and returned to room temperature after their intended exposure period. Mortality was not determined in any treatments (tests or control) of a test temperature until the replications assigned to the longest treatment had been removed from the exposure chamber. This design was used because it allowed one control treatment to be used for each test temperature, thereby keeping the number of larvae needed manageable. Previous unpublished work by the senior author had shown that there was no significant difference between results when mortality due to low temperature exposures was assessed either 2 hr or 24 hr after exposure completion.

15. For temperatures above freezing, control and test treatments that had been removed from the exposure chamber were transferred to fresh plant material every 2 days until the last treatment group was removed. Mortality was then assessed in these groups 98 hr after exposure initiation. For below-freezing temperatures, mortality was assessed 24 hr after test initiation. In all tests, larvae were considered dead if they did not respond when prodded with a camel brush.

Data analysis

16. Initially, uniformity trials were conducted to ensure that no significiant differences existed among replications of the same treatment (Hewlett and Plackett 1979). If no significant difference was found, replicate results were summed resulting in a composite mortality value for each treatment. These values were then screened, and any temperature groups with a corresponding control mortality greater than 10 percent were deleted from the analysis (Hewlett and Plackett 1979). For tests with control mortality less than 10 percent, a weighting coefficient w was calculated. Multiplying this

Table 11

Analysis of Residuals for Minimum Logit Chi-Square Analysis

of Mortality for Tests Conducted at -6°C

Exposure Time	No. Dead (Test)	Logit (Estimated)	Percent Dead (Estimated)	No. Dead (Estimated)	Standardized Residual	Std. Res.^{2}
1	4	-1.60	16.8	10.08	-2.10	4.40
2	12	-1.21	23.0	13.80	-0.55	0.30
4	26	-0.43	39.4	23.64	0.62	0.38
8	48	1.13	75.6	45.36	0.79	0.62
16	58	4.25	98.6	59.16	-1.27	1.61
						7.31 n.s.*

* n.s. denotes nonsignificance for Pearson's statistic at $P = 0.05$.

Table 9

Analysis of Residuals for Minimum Logit Chi-Square Analysis

of Mortality for Tests Conducted at -2°C

Exposure Time	No. Dead (Test)	Logit (Estimated)	Percent Dead (Estimated)	No. Dead (Estimated)	Standardized Residual	Std. Res.^{2}
1	3	-3.52	2.9	1.74	0.97	0.94
2	2	-3.24	3.8	2.28	-0.19	0.04
4	3	-2.68	6.4	3.84	-0.44	0.19
8	9	-1.56	17.4	10.44	-0.49	0.24
16	40	0.68	66.6	39.96	0.01	0.00
						1.41 n.s.*

* n.s. denotes nonsignificance for Pearson's statistic at P = 0.05 .

Table 10

Analysis of Residuals for Minimum Logit Chi-Square Analysis

of Mortality for Tests Conducted at -4°C

Exposure Time	No. Dead (Test)	Logit (Estimated)	Percent Dead (Estimated)	No. Dead (Estimated)	Standardized Residual	Std. Res.^{2}
1/2	1	-2.58	7.1	4.26	-1.64	2.68
1	5	-2.37	8.5	5.10	-0.05	0.00
2	9	-1.95	12.4	7.44	0.61	0.37
4	14	-1.12	24.6	14.76	0.23	0.05
8	38	0.55	63.3	37.98	0.00	0.00
						3.10 n.s.*

* n.s. denotes nonsignificance for Pearson's statistic at P = 0.05 .

Table 7

Estimated Line Parameters of the Proposed Regression Equations Generated by the Analysis for Each Test Temperature

Temperature, °C	Logit-Intercept	Slope*	LT-50, hr*
+2	-1.08	0.07 ± 0.016	16.00 ± 2.146
-2	-3.79	0.28 ± 0.070	13.68 ± 1.442
-4	-2.79	0.42 ± 0.124	6.69 ± 0.820
-6	-2.07	0.40 ± 0.094	5.21 ± 0.830

* Presented as estimated value ±2 S.E.

Table 8

Analysis of Residuals for Minimum Logit Chi-Square Analysis of Mortality for Tests Conducted at +2°C

Exposure Time	No. Dead (Test)	Logit (Estimated)	Percent Dead (Estimated)	No. Dead (Estimated)	Standardized Residual	Std. Res.^2
3	14	-0.87	29.5	17.70	-1.05	1.10
6	17	-0.66	34.1	20.46	-0.94	0.89
12	30	-0.24	44.0	26.40	0.94	0.89
24	40	0.60	64.6	38.76	0.34	0.11
48	55	2.28	90.7	54.42	0.26	0.07
96	59	5.64	99.6	59.76	-1.55	2.41
						5.47 n.s.*

* n.s. denotes nonsignificance for Pearson's statistic at $P = 0.05$.

Table 5

Principal Values Used in Calculations for Logit Analysis of -4°C Test Results*

Exposure Time, hr	No. Tested	No. Dead (Test)	Percent Dead (Test)	Percent Dead (Control)	Logit (Observed)	Weighting Coefficient	Logit (Estimated)
1/2	60	1	2	2	-4.08	0.01	-2.58
1		5	8		-2.40	0.06	-2.37
2		9	15		-1.73	0.11	-1.95
4		14	23		-1.19	0.16	-1.12
8		38	63		0.53	0.22	0.57

* For each exposure time (treatment), replications have been summed and values represent composite results.

Table 6

Principal Values Used in Calculations for Logit Analysis of -6°C Test Results*

Exposure Time, hr	No. Tested	No. Dead (Test)	Percent Dead (Test)	Percent Dead (Control)	Logit (Observed)	Weighting Coefficient	Logit (Estimated)
1	60	4	7	2	-2.64	0.05	-1.60
2		12	20		-1.39	0.15	-1.21
4		26	43		-0.27	0.23	-0.43
8		48	80		1.39	0.15	1.13
16		58	97		3.37	0.03	4.25

* For each exposure time (treatment), replications have been summed and values represent composite results.

Table 3

Principal Values Used in Calculations for Logit Analysis of +2°C Test Results*

Exposure Time, hr	No. Tested	No. Dead (Test)	Percent Dead (Test)	Percent Dead (Control)	Logit (Observed)	Weighting Coefficient	Logit (Estimated)
3	60	14	23	5	-1.19	0.14	-0.87
6		17	28		-0.93	0.16	-0.66
12		30	50		0.00	0.22	-0.24
24		40	67		0.69	0.20	0.60
48		55	92		2.40	0.07	2.28
96		59	98		4.08	0.01	5.64

* For each exposure time (treatment), replications have been summed and values represent composite results.

Table 4

Principal Values Used in Calculations for Logit Analysis of -2°C Test Results*

Exposure Time, hr	No. Tested	No. Dead (Test)	Percent Dead (Test)	Percent Dead (Control)	Logit (Observed)	Weighting Coefficient	Logit (Estimated)
1	60	3	5	2	-2.94	0.04	-3.52
2		2	3		-3.37	0.02	-3.24
4		3	5		-2.94	0.04	-2.68
8		9	15		-1.73	0.11	-1.56
16		40	67		0.69	0.21	0.68

* For each exposure time (treatment), replications have been summed and values represent composite results.

Table 1

Duration of Exposure (Hours) to Each of the Low Temperatures Tested

Test Temperature					
-6°C	-4°C	-2°C	+2°C	+4°C	+6°C
1.0	0.5	1.0	3.0	3.0	3.0
2.0	1.0	2.0	6.0	6.0	6.0
4.0	2.0	4.0	12.0	12.0	12.0
8.0	4.0	8.0	24.0	24.0	24.0
16.0	8.0	16.0	48.0	48.0	48.0
--	--	--	96.0	96.0	96.0

Table 2

Results of Uniformity Trials Within Treatments for Exposures to +6°, +4°, +2°, -2°, -4°, and -6°C

Expsoure	Chi-Square Values*					
Time, hr	+6°C	+4°C	+2°C	-2°C	-4°C	-6°C
Control	0.54	0.44	2.11	2.03	2.03	2.03
0.5	--	--	--	--	2.08	--
1.0	--	--	--	4.21	4.16	2.14
2.0	--	--	--	1.04	2.08	0.63
3.0	2.03	2.03	5.21	--	--	--
4.0	--	--	--	4.21	4.44	0.63
6.0	2.03	0.00	4.67	--	--	--
8.0	--	--	--	0.78	0.14	1.88
12.0	0.00	2.03	2.80	--	--	--
16.9	--	--	--	1.05	--	1.05
24.0	0.00	4.16	2.85	--	--	--
48.0	2.11	2.11	5.70	--	--	--
96.0	2.11	0.54	2.08	--	--	--

* The critical Chi-Square value at $P = 0.05$ is 5.991.

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Recommendations

41. Because LT-50 values were not obtained for temperatures above +2°C, there is no indication of population survival after exposures of long durations to these temperatures. Since it was noted that larvae did not feed during laboratory exposures, mortality could result from starvation at these temperatures. Also, no movement of larvae was noted in these treatments. If larvae are not able to leave decaying plant material, mortality may result from drowning.

42. The need for further research on this subject is greatly indicated by these results. Tests should be conducted on other instars to elucidate the relationship between surface:volume ratios of larvae and tissue freeze susceptibility. The role of acclimation in preparing larvae for cold exposure should also be investigated. The significance of the reported increased body size at low rearing temperatures (Center and Durden 1981) should be studied to determine if these individuals are less susceptible to starvation than are smaller, warm-acclimated individuals. This is a very important consideration since available food supply is greatly reduced by frost damage during early winter. Also, the significance of the nonfeeding behavior of larvae exposed to low temperatures, possibly increasing frost resistance, should be studied.

43. Before such tests can be initiated, attention should be directed at improving rearing techniques of *S. albiguttalis*. If laboratory rearing success cannot be improved, future tests of the above nature should be conducted at a facility in close proximity to an established field population of this moth.

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exposures by causing a decrease in food material in the digestive tract.

35. Comparison of estimated mortality between the below-freezing and the +2°C regression equations cannot be made directly. Although initial exposures to +2°C are seemingly more lethal than exposures to below-freezing temperatures (Figure 4), this interpretation is probably a result of inconsistency in the experimental design. If treatments to the below-freezing temperatures had been returned to control conditions for approximately 4 days prior to recording results, as was the procedure for the +2°C test, mortality would probably have increased significantly in the shorter exposure treatments, resulting in an increase in the logit intercepts.

Summary and Recommendations

Summary

36. Laboratory tests were conducted to develop models for predicting mortality of the third larval instar of *Sameodes albiguttalis* (Warren) resulting from exposure to constant low temperatures in the laboratory.

37. Mortality resulting from exposures to +6° or +4°C was less than mortality in corresponding control treatments. It is suggested that prior acclimation to these temperatures may increase survival of larvae at the below-freezing temperatures.

38. Exposures to +2°C were, however, shown to cause mortality. The data indicated that greater than 25-percent mortality occurred after exposures of less than 2 hr if the results were not taken until 98 hr after exposure initiation. Similarily, 90-percent mortality resulted after only 48 hr exposure to this temperature.

39. Results of tests performed at -4° and -6°C were statistically indistinguishable. Twenty-five-percent mortality at these temperatures was estimated after 2 to 4 hr exposure. Ninety-five-percent mortality was estimated to occur after 9 to 11 hr exposure.

40. The regression equation for mortality at -2°C had a similar slope value to those equations calculated for -4° and -6°C; mortality at this temperature may have resulted from tissue freeze damage. Exposures to these temperatures, however, are estimated to require an increase in duration of two to three times that of either -4° or -6°C exposures before the same level of mortality is reached.

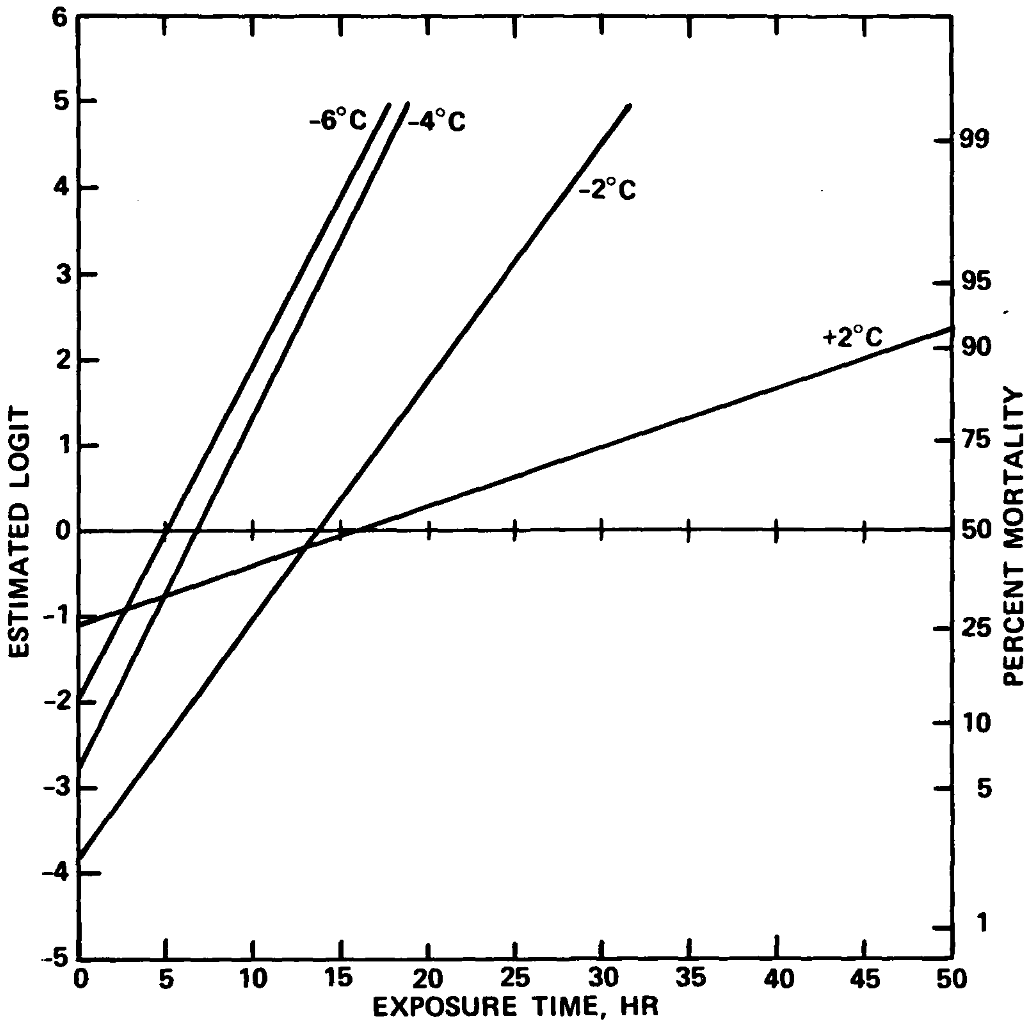

Figure 4. Predicted mortality of third instar *S. albiguttalis* larvae resulting from exposures to +2°, -2°, -4°, and -6°C

(a) the supercooling point of body fluids, (b) the body size, (c) the duration of exposure to a freezing temperature, and (d) the presence of inoculating agents, such as food particles in the gut.

34. Because larvae were feeding immediately prior to exposure to the test temperatures, it is possible that ingested food particles enhanced ice formation in gut tissues. Interestingly, larvae did not feed during exposures to the less lethal, nonfreezing temperatures. Also, 96-hr exposures to +6° and +4°C resulted in less mortality than corresponding control conditions. It is possible, since feeding is halted, that preconditioning larvae to these temperatures may increase their survival to subsequent below-freezing

28. Calculated values of Pearson's statistic are also given in Tables 8-11. These values show acceptable overall agreement between the fitted lines and the observed data for -2°, -4°, and -6°C tests $\left(X^2_{0.05,3} = 7.815\right)$ and for the +2°C test $\left(X^2_{0.05,4} = 9.488\right)$. The critical value was greater for the +2°C test because more exposure durations were tested for this temperature (Table 1).

Discussion

29. Results indicate that short-term exposures to temperatures of +6° and +4°C were tolerated by third instar larvae of *S. albiguttalis*. However, these temperatures would likely be detrimental if exposure durations were increased because larvae apparently did not feed even in the 96-hr treatments.

30. The high mortality resulting from exposures to +2°C suggests that the minimum tolerance limit is between +2° and +4°C. Danks (1978) discusses various theories of cold-injury at temperatures above freezing but below the normal range for development. Exposure to this temperature may have altered essential biochemical pathways by lowering reaction rates via insufficient activation energy or by altering the chemical bonding of constituent reactants.

31. Results of tests at below-freezing temperatures show that mortality is dependent upon the actual exposure temperature and upon the duration of exposure to that temperature. The fitted lines to the -4° and -6°C results are essentially parallel (Figure 4). Although there were approximately 1.5 hr difference in estimated LT-50 values for these two temperatures, this difference was statistically nonsignificant (±2 S.E.) (Table 7).

32. At -2°C, a twofold increase in exposure duration, as compared with -4° and -6°C models, was required to cause 50-percent mortality. Generally, this relationship was similar for mortality above and below the 50-percent level (Figure 4). It can, therefore, be concluded that after equal exposure durations, approximately twice the number of *S. albiguttalis* larvae are killed at either -4° or -6°C as are killed at -2°C.

33. Since slopes are similar at the three below-freezing temperatures (Table 7), it is probable that death was resulting from similar factors and simply required a longer exposure duration for initiation at -2°C. An obvious possibility is tissue freeze damage. Salt (1953, 1956, 1957, and 1966) and Asahina (1969) have shown that tissue freezing in insects is influenced by

difference was to be expected because results were not recorded for the +2°C tests until 98 hr after test initiation, whereas results of the below-freezing tests were recorded 24 hr after test initiation.

25. Table 7 gives the logit-intercepts, slopes, and estimated LT-50 values calculated by the analyses. For the below-freezing tests, the logit-intercepts increased with decreases in test temperature. This shows a decrease in tolerance to initial exposures as temperatures decreased below freezing. Also, even though LT-50 values were not calculated for +4° and +6°C, initial mortality was evidently greater at +2°C than both +4° and +6°C. Concluding that short exposures (i.e. less than 10 hr) to +2°C are more lethal to *S. albiguttalis* larvae than are similar exposures to below-freezing temperatures, however, would be unjustified, even though the +2°C regression equation had the highest logit-intercept. Had mortality in the below-freezing tests been recorded 98 hr after exposure initiation, as was the case for +2°C, mortality would have probably been considerably greater.

26. Differences in slope were nonsignificant (i.e. less than ±2 S.E.) (Table 7) among the below-freezing temperature regression equations, even though the increase in observed mortality due to increase in exposure time was actually much more abrupt at -2°C (Table 4) than at -4° and -6°C. Estimated LT-50 exposure times decreased with decreases in temperature. Values were statistically similar (Table 7) at -4° and -6°C. Exposure to -2°C, however, required approximately a twofold increase in duration to result in 50-percent mortality. The LT-50 exposure time for the +2°C model was 16 hr. Although this value is not directly comparable to LT-50 values for the below-freezing tests, it is interesting that exposures of six times this duration (96 hr) to +4° and +6°C resulted in less than 10-percent mortality.

27. Tables 8-11 present the results of tests comparing the fitted lines with the observed data. The 1-hr exposure to -6°C was the only treatment with a standarized residual exceeding 2.0 in absolute value. As can be seen in Table 11, the proposed model provides an estimated mortality which greatly exceeds the observed mortality for this treatment. Possibly, actual temperature in these "test" petri dishes did not drop as rapidly as the monitored temperatures in the exposure chamber. If so, the difference between true versus assumed exposure time would have been proportionally greater in this shortest exposure treatment and could result in the observed overestimation of mortality.

To find P , the proportion of larvae expected to be killed by a given exposure duration, the estimated logit response L was calculated from the regression formula and was transformed to a proportion by tables. Conformance to the assumed linear relationship was questioned for a given treatment if d had an absolute value greater than 2.0 (Ashton 1972, Hewlett and Plackett 1979).

21. To test the overall fit of the regression line, Pearson's statistic was calculated by summing the squares of the standarized residuals of all treatments for a given temperature (Hewlett and Plackett 1979). The fit was rejected if this value exceeded the tabled chi-square value at $p = 0.05$ for the appropriate degrees of freedom.

Results

22. Variation in the number of larvae killed among replications of the same treatment was nonsignificant for all test temperatures (Table 2). Replicate mortality results were, therefore, summed and these values were analyzed by the Minimum Logit Chi-Square procedure.

23. The composite mortality values from tests conducted at +6° and +4°C were, however, excluded from further analyses because exposures to the longest time duration (96 hr) at these temperatures resulted in mortality values which were too low (<10 percent) for estimation of LT-50 values. At +6°C only three larvae were killed by the 96-hr exposure, while four were killed by the same length of exposure to +4°C. Additional tests of longer duration have not been conducted because of the unavailability of larvae. Also, in tests of sufficient duration to result in test mortality greater than 50 percent at these two temperatures, corresponding control mortality will probably exceed the 10 percent analysis guideline and invalidate the results. Control mortalities for the +6° and +4°C (four and five dead larve, respectively) were, in fact, higher than in 96-hr test treatments.

24. Principal values used in the analyses for tests conducted at +2°, -2°, -4°, and -6°C are given in Tables 3-6, respectively. Definition of the variables was given in the Materials and Methods section. The number of dead larvae gradually increased with increase in exposure time for all temperatures except -2°C (Table 4). Control mortality, highest in the +2°C test (5 percent), was only 2 percent in each of the below-freezing tests. This

value by the number of test organisms in the corresponding treatment provided a means of negating the influence of inherent (control) mortality on experimental results (Hewlett and Plackett 1979).

17. Results for each temperature were then analyzed by the Minimum Logit Chi-Square procedure as described by Ashton (1972) and Hewlett and Plackett (1979). This required transforming the percent mortality values to logits ℓ by the equation:

$$\ell = \log_e\left(\frac{p}{1 - p}\right) \tag{1}$$

where p = proportion of dead larvae in the test treatment. These values were then plotted against exposure time (hours) and a weighted regression analysis was performed which fitted a straight line to these points.

18. The fitted lines allow logit estimates L of the mortality response for any exposure duration and had the general form:

$$L = a + bx \tag{2}$$

where

a = logit intercept of the fitted line

b = slope of the fitted line

x = exposure time for a given treatment

19. The LT-50 exposure time H (i.e. the time resulting in 50-percent mortality) was then estimated by the equation:

$$H = -\frac{a}{b} \tag{3}$$

Methods for calculating standard errors for the slope and LT-50 values are given by Ashton (1972) and Hewlett and Plackett (1979).

20. For each fitted line, a "goodness of fit" test was conducted to determine the adequacy of the calculated model to the observed results (Hewlett and Plackett 1979). Initially, a standardized residual d was calculated for each treatment group to locate if the results of any of the treatments violated the assumptions of linearity. The equation used was:

$$d = \frac{r - nP}{nP(1.0 - P)^{1/2}} \tag{4}$$

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